IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 7, Issue 2, July 2021
Impact Factor: 5.731

Efficacy of *Achromobacter Spp.* Isolated from Rhizosphere of Soybean Against *Macrophomina phaseolina*

Kriti Joshi[1] and Naveen Joshi[2]
Department of Biotechnology and Microbiology[1,2]
SJHS Gujarati Innovative College of Commerce and Science, Indore, Madhya Pradesh, India
kriti_dave07@yahoo.co.in and naveenjoshi44@gmail.com

Abstract: *Soybean is an important oil seed crop produced in India, Madhya Pradesh is the largest soybean producing state. Charcoal rot caused by Macrophomina phaseolina is a major threat to soybean production leading to 30-50% yield loss. Biological control is an effective measure for management of this disease. Soil rhizosphere is a rich source of beneficial microbes able to suppress plant pathogens and promote plant growth. Present study aimed at isolating and characterizing indigenous antagonistic bacteria against M. phaseolina causing charcoal rot in soybean.*

Keywords: Soybean, *Achromobacter, Macrophomina phaseolina*, Biological control, Charcoal rot

I. INTRODUCTION

Oil seed crop *soybean Glycine max* (L.) is an important crop produced in India. Madhya Pradesh is the largest soybean producing state (Soybean Outlook, 2019). One of the major threats to soybean production is soil borne pathogen *Macrophomina phaseolina* (Tassi) Goid. It infects many crops viz. soybean, cotton, maize, mungbean, sorghum, chickpea etc. (Dave *et al.,* 2020). Charcoal rot caused by *M. phaseolina* in soybean has emerged as an important disease leading to 50% loss. *M. phaseolina* remains in soil as sclerotia for longer duration. Being a saprophytic fungus; its control is difficult. Chemical fungicides are toxic, pollute the environment, kill beneficial microbes in the soil and are expensive. As a result, biological control is an effective measure to reduce disease incidence and improve crop production. Rhizospheric bacteria have intrinsic properties enabling them to control phytopathogens and also promote plant growth (Raaijmakers *et al*., 2009). Biological control has many advantages over chemical control *viz*. ecofriendly approach, nontoxic and long-term sustainability. The present study was carried out to isolate potential antagonistic bacteria from rhizosphere of soybean against *M. phaseolina*. Isolate K06 was found to be an effective antagonist; showing 49% inhibition of *M. phaseolina*. On the basis of gram staining and biochemical characteristics it was observed that probably K06 belongs to *Achromobacter sp.* The results of 16srRNA sequencing reveal 99% homology with *Achromobacter xylosoxidans.* Studies have shown the bacteria belonging to *Achromobacter* have plant growth promoting attributes. The isolate K06 can be further evaluated for production of antifungal compounds responsible for antagonism. It proves to be an effective alternative strategy, can be used for increasing crop production.

II. MATERIALS AND METHOD

2.1 Soil Sample Collection

The soil samples were collected form rhizosphere of healthy soybean, growing in agricultural field of Indore, M.P. India. The samples were transferred in laboratory and dried.

2.2 Isolation and Purification of Bacteria

The bacteria were isolated using serial dilution method (Dubey and Maheshwari, 2022). 1 g of soil sample was dissolved in sterile distilled water tube (10 ml) and serial dilution was made up to 10^{-8} dilutions by transferring 1 ml of sample from each preceding tube in fresh sterile distilled water tube (9 ml). The serially diluted samples (10^{-4}, 10^{-5} and 10^{-6}) were plated on nutrient agar plates (0.1 ml) and incubated for 24 hrs. at 28^{0}C. Single colonies were obtained are transferred on fresh nutrient agar plates and purified using sector plate method.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 7, Issue 2, July 2021
Impact Factor: 5.731

2.3 Antagonistic Activity

Antagonistic activity of isolated bacteria against *M. phaseolina was* evaluated *in-vitro* by dual culture technique (Ramanathan *et al.,* 2002).

2.4 Dual Culture Technique

Isolates were screened on the basis of their ability to inhibit *M. phaseolina* using dual culture technique explained by Morton and Stroube (1955), with optimization and Singh *et al.,* (2008). The pathogenic fungi mycelial disc and isolates were placed on equidistance from each other in a PDA plate. Experiment was carried out in triplicate and plate with pathogen served as control. Plates were incubated for 6 days at 28^0C. Radial growth of *M. phaseolina* was calculated in control and test plate, percentage inhibition was calculated using formula given by Vasebi, (2008).

2.5 Morphological and Biochemical Characterization

A. Gram Staining

Gram staining of screened isolates was performed with 24 hrs. old culture. Smear was prepared on clean glass slide and heat fixed. Crystal violet was applied for 1-2 minutes and after washing grams iodine was applied for 1 min. followed by washing with 95% alcohol and water. Saffranin was applied for 5 min. and followed by washing. Air dried slides were observed under oil immersion lens.

B. Biochemical Tests

For biochemical identification various biochemical tests were performed *viz.* Catalase test, Oxidase test, Indole test, MR, VP, Citrate Utilization, Gelatin hydrolysis, Starch hydrolysis, and TSI. The entire tests were performed according to Cappuccino and Sherman (2006).

C. Molecular Characterization

The amplification of DNA was performed using 16S rRNA universal primers. The amplified DNA products were sent for 16S rRNA sequencing to NCIM Pune (Sanger sequencer was used). The sequences obtained were searched for closest sequences and phylogenetic analysis was done using MEGA-X (64bit) software.

III. RESULTS AND DISCUSSION

3.1 Soil Sample Collection and Isolation of Bacteria

Soil samples were collected in sterile bags and transported to laboratory for further studies. Samples were dried and used for isolation of bacteria. A total of 35 bacterial morphotypes were isolated from the rhizospheric soil sample of soybean plants grown in agricultural field of Indore M.P. India. Isolates were purified by quadrant streaking and stored at 4^0C in a refrigerator. Rhizosphere is the source of beneficial bacteria with the capability to control plant pathogen and growth promotion. The bacteria isolated from the same environmental conditions have efficiency to control pathogen as they are well versed with the environment. The ability of indigenous bacteria to adopt increases their probability to successfully control the disease incidence and enabling their potential to use as effective formulation for disease control and growth of plant (Dave *et al.,* 2020).

3.2 Antagonistic activity (Dual culture Technique)

All the 35 obtained isolates were screened for antagonistic activity against *M. phaseolina* using dual culture technique. Among the 35 isolated bacteria 5 isolates were screened namely K06, K24, K35, K30 and K02 on the basis of their ability to inhibit pathogenic fungi, the percentage inhibition was depicted in Figure 1. Isolate K06 (49%) showed maximum percentage inhibition against *M. phaseolina* in *in-vitro* conditions followed by K35 (44%), K24 (40%), and K30 (35%) lowest by isolate K02 (33%).

The ability of isolates to inhibit the growth of *M. phaseolina* demonstrates the presence of indigenous bacteria with antagonistic activity in the rhizosphere of soybean. The rhizosphere of soil is rich source of beneficial microbes having potential to formulate biocontrol agent (Kumar *et al.,* 2008). Dave *et al.,* (2020) isolated bacteria with antagonistic potential form rhizosphere soil of soybean. Findings of the study demonstrate the potential of indigenous bacteria in controlling pathogens.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 7, Issue 2, July 2021
Impact Factor: 5.731

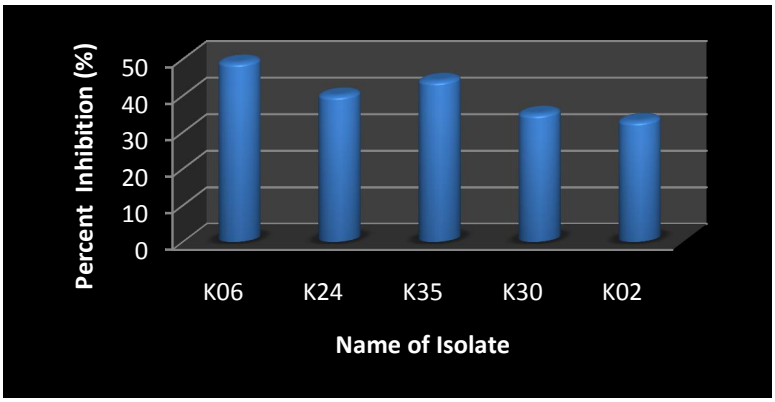

Figure 1: Percentage inhibition of five potential isolates

3.3 Morphological and Biochemical Characterization

The morphological studies revealed that all the five isolates are bacilli in shape; all the isolates are gram positive in nature except K06 which is gram negative. The K06 was found to be motile on checking motility. The biochemical analysis of all the five isolates is depicted in the table 1. All the five isolates have the ability to produce catalase enzyme as they showed positive catalase reaction. All the isolates were oxidase negative except K06 (Positive). Similarly only isolate K06 has the ability to produce IAA in broth media and showed positive indole test; other four isolates were indole negative. All the five isolates have the ability to ferment glucose and showed positive MR and VP test. All the five isolates are capable of gelatinase as all showed gelatin hydrolysis after two days. Isolates showed positive TSI test, resulted due to fermentation of sugars and production of acid. The biochemical and morphological studies indicates that K06 belongs to *Achromatobacter sp,* whereas K02, K30, K35 and K24 belong to *Bacillus Sp.*

Table 1: Biochemical Characters of isolates

S. No.	Biochemical Test	K02	K06	K24	K30	K35
1	Catalase	P	P	P	P	P
2	Oxidase	N	P	N	N	N
3	Citrate Utilization	N	N	N	N	N
4	Indole	N	P	N	N	N
5	MR	P	P	P	P	P
6	VP	P	P	P	P	P
7	Gelatin Hydrolysis	P	P	P	P	P
8	TSI	Acidic but alkaline slant and gas production	Acidic slant and acidic but	Acid but, alkaline slant	Acid but, alkaline slant	Acid but, alkaline slant and H_2S Production
9	Starch Hydrolysis	P	P	P	P	P

P: Positive, N: Negative

3.4 Molecular Characterization

Isolate K06 showed maximum percentage inhibition and has the remarkable difference form other isolates, thus16S rRNA sequencing was done for isolate K06. Result of 16S rRNA sequencing showed that K06 shared 99% homology with *Achromobacter xylosooxidans* strain NBRC 15126. Tree for the same was constructed using neighbor joining method (Figure 2). Present study shows the potential of *Achromobacter xylosooxidans* as effective BCA against *Macrophomina phaseolina*.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 7, Issue 2, July 2021
Impact Factor: 5.731

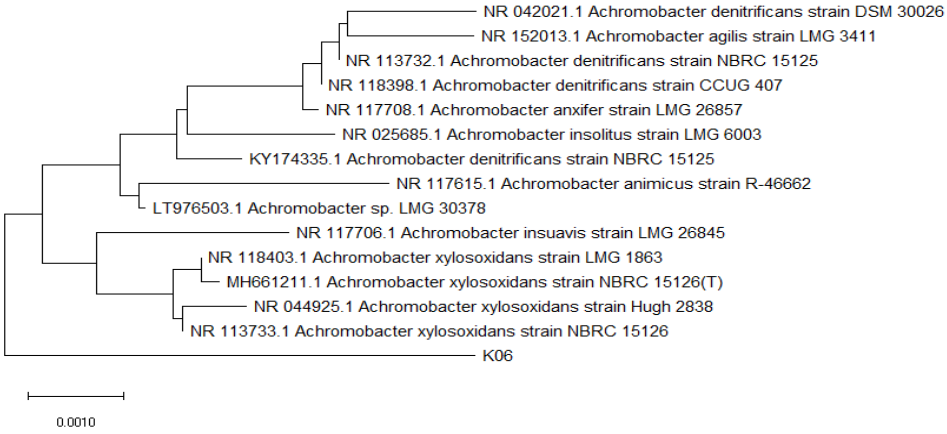

Figure 2: Phylogenetic tree of K06

IV. CONCLUSION

The present study unveils the potential of *Achromobacter xylosoxidans* as effective BCA against *M. phaseolina.* It can be further studied for antifungal metabolites involved in antagonism along with PGPR traits. The use of BCAs is an ecofriendly and sustainable approach for disease management as compared to hazardous chemical control.

REFERENCES

[1]. Agricultural Market Intelligence Centre, PJSTAU, Soybean Outlook (2019): Pg. 2.

[2]. Cappuccino, J. C. and Sherman, N. (2006) Microbiology: A Laboratory Manual. Sixth Edition, Benjamin Cummings Publishing, USA, pp: 153-155; 165-167.

[3]. Dave K, Gothalwal R, Singh M, Joshi N (2020) Facets of rhizospheric microflora in biocontrol of phytopathogen *Macrophomina phaseolina* in oil crop soybean. Archives of Microbiology DOI: 10.1007/s00203-020-02046-z.

[4]. Dave K, Singh M, and Gothalwal R, (2020) Antagonistic efficiency of Indigenous Rhizospheric Bacteria agains *Macrophomina phaseolina* causing Charcoal Rot in Soybean. Res. Jr. of Agri. Sci., 12(2): 470-473.

[5]. Dubey R.C. and Maheshwari D.K. (2002) Practical Microbiology. S Chand Co. New Delhi India pp:184-205.

[6]. Kumar A, Saini P, Shrivastava JN. 2008. Production of peptide antifungal antibiotic and biocontrol activity of Bacillus subtilis. Indian Jr. Exp. Biology 47: 57-62.

[7]. Morton D.J and Stroube W.H. (1955) Antagonistic and stimulatory effects of soil microorganisms upon *Sclerotium rolfsii*. Phytopahtology, 45: 417-420.

[8]. Raaijmakers J.M., Paulitz T.C, Steinberg C., Alabouvette C. and Moenne- Loccoz Y. (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321: 341-361. DOI: 10.1007/s11104-008-9568-6.

[9]. Ramanathan,A.,Shanmugam,V., Ragachander T. and Samiyappan,R.,(2002):Induction of Systemic Resistance in Ragi against Blast Disease by Pseudomonas fluorescens. Ann. Pl. Protec.Soc., 10 (2) :313-318.

[10]. Singh N., Pandey P, Dubey R.C. and Maheshwari D. K. (2008) Biological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* (Sarg.) by rhizosphere competent *Bacillus subtilis* BN1. World J Microbiol Biotechnol (2008) 24:1669–1679. DOI: 10.1007/s11274-008-9680-z.

[11]. Vasebi Y. (2008) Biological control of soybean charcoal rot caused by *Macrophomina phaseolina* using antagonistic bacteria. Master's Dissertation. Tarbitat Modares University, Tehran pp. 132.